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(54) Title: METHOD FOR REMOVAL OF HUMAN HORNY TISSUES

(57) Abstract

This invention relates to a composition and a method to facilitate physical trimming and removing horny human tissues in a speedy and atraumatic fashion. Particularly, the invention includes a composition which softens horny human tissue comprising an effective amount of at least one sulfur containing compound. In addition, the invention includes a method of softening horny human tissues comprising applying an effective amount of a composition comprising at least one sulfur containing compound to a human for a duration of time sufficient to soften. Still further, the invention contemplates a method for removing horny human tissue comprising applying a composition comprising an effective amount of at least one sulfur containing compound to a human for a duration of time sufficient to soften and removing said horny tissue by a physical means. Still further the invention contemplates a kit which comprises a composition which softens horny human tissue comprising an effective amount of at least one sulfur containing compound and at least one active agent useful in the treatment of horny human tissue.

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# METHOD FOR REMOVAL OF HUMAN HORNY TISSUES

## FIELD OF THE INVENTION

This invention relates to a novel method to soften, and subsequently to facilitate the removal human horny tissues, i.e., nail, callus, and corn, using sulfur containing compounds. This method is particularly useful in atraumatic removal of the diseased portion of fungi-infected nails prior to topical treatment.

## BACKGROUND OF THE INVENTION

Onychomycosis is a fungal disease of the human nail. The symptoms of this disease are a split, thickened, hardened, and rough nail plates. This is caused by any of a number of organisms and is particularly prevalent in the elderly. Typically fungal infections are treated by topical application of antifungal agents and/or oral administration of antifungal agents. Unlike other fungal infections, there is no topical treatment for onchomycosis which is approved by the United States Food and Drug Administration. It is desirable to treat this disease topically due to the potential for side effects that have been associated with some of the oral treatment regimens. One reason for the absence of a topical treatment is that in this disease, the symptomatic thickened nail plate prevents topical agents from reaching the site of the infection.

The human nail plate is comprised of hard nail keratin with a high content of disulfide-linkeages (cystine), and is approximately 0.5 mm thick for finger nails, and about 1.5 mm thick for big toe nails. A nail plate grows out from the nail matrix, a living and highly proliferative epithelial tissue under the eponychium, and overlays the nail bed, a soft and normally non-cornified tissue. Although it appears visually uniform, a nail plate is made of at least two discernible macroscopic strata, i.e., the dorsal nail plate and intermediate

nail plate. The dorsal nail plate is harder and thinner than the intermediate nail plate. The thick nail plate and its dense keratin nature present a formidable barrier for topical drugs to penetrate, and accounts for the failure of many topical antifungal agents to treat fungal infected nails.

Clinical evidences show that complete removal, partial removal, or thinning of a diseased nail plate prior to topical drug application improves the efficacy of topical onychomycosis treatment. One study described drilling a small array of holes in a patient's toenail prior to treatment with an antifungal agent. (Brem, J., Curtis, 1981, 27:69-76). However, in many cases physical removal or thinning of the nail is hampered by the fact that fungus infected nails are often significantly thickened by the disease. For example, among the patients involved in the Brem study, onychomycosis resulted in a nail thickness of 5 mm for a small toe nail, and 9 mm for big toe nails. Patients were unable to cut them, and needed to use a file to have them trimmed. Unfortunately, this procedure was quite painful due to the underlying condition and the pressure necessary to trim the nails. This type of physical procedure is often particularly difficult for elderly who suffer from this disease and are often not flexible enough to reach their affected nails, let alone to physically trim them.

Aside from physical methods of breaking through the diseased nail plate, chemical methods have been used, followed by antifungal therapy. (Rollman, O. Dermatologica, 1982, 165:54-61). For example, a combination of urea and salicylic acid were used for non-surgical removal of diseased nails, followed by antifungal therapy. (South, D.A. and Farber, E.M., CUTIS, 1980, 25:609-612; Buselmeier, T.J., CUTIS, 1980, 25:397-405). The urea concentrations used by these investigators ranged from 20% to 40%, and salicylic acid 10% in a hydrophobic base. It took about one week under occlusion to accomplish the dystrophic nail avulsion (removal of the nail).

Others have combined antifungal agents with chemical agents to

remove the diseased nail plate and treat onychomycosis. (Hardjoko et al.,"Treatment of onychomycosis with a bifonazole-urea combination", Mycoses, 1990, 33,4:167-171) In one clinical trial, a combination of 1% bifonazole and 40% urea ointment were used. The combination ointment was applied to the fungus-affected nail with an occlusive dressing every day for two weeks, followed by 1% bifoconazole cream topically for one month. Before changing the dressing, the affected nail was immersed in warm water for about 10 minutes to facilitate easy removal of the nail. Then the nail and nail bed were scraped with a plastic nail file.

Aside from the aforementioned methods of treating the diseased nail plate, others have attempted to treat a diseased nail plate without removing the diseased nail plate. One treatment incorporates certain mercaptans with antifungal agents to treat a diseased nail. (Morimoto, JP 8231430 A, JP 95-41722). The incorporated mercaptans include 4-t-butylbenzyl mercaptain, thiosalicylic acid, cysteamine hydrochloride, or thioglycolic acid and its derivatives such as ammonium thioglycolate, or butyl thiglycolate. In this disclosure, the mercaptan compound is directly incorporated in the drug formulation.

Olthoff et al. In EP 440,298, disclose the use of sulfur containing amino acid derivatives, dithiothreitol, and ammonium thioglycolate in topical preparations for the treatment of human nails.

U.S. Patent 5,696,164 (Sun et al., 1997) discloses the use of sulfur containing amino acids and amino acid derivatives, such as cysteine and N-acetyl cysteine, and urea to increase drug permeability in a nail plate. The sulfhydryl (SH) compounds in the aforementioned patent disclosures are used as nail penetration enhancers to increase drug permeability in the nail.

A US patent application (US Serial No. 60/080,212) filed by Sun et al., describes the use of sulfur containing compounds such as calcium

thioglycolate as chemical nail penetration enhancers to improve topical drug delivery into the nail. The method of nail penetration enhancement is a two-step process: (a) pretreating a nail plate first with a composition comprised of certain sulfur containing compounds; and (b) applying a topical composition containing active agents to the pretreated nail plate. In all these disclosures involving the use of nail penetration enhancer to improve topical drug delivery, the nail plate remains intact while its permeability is altered to facilitate the permeation of the active agent.

The teachings of Morimoto, Olthoff, and Sun show a method of treating onychomycosis using a penetration enhancer and an antifungal agent. However they do not teach a method of softening diseased nails to the consistency which allows for easy removal of the diseased nail prior to treatment with an antifungal agent.

In addition to diseased nails, other human horny tissues, such as ingrown nail, corns and callus, also require trimming to eliminate pain or discomfort. Certain chemicals are used to facilitate removal of the undesirable horny tissues. Salicylic acid is commonly used to remove the corns and callus. Sodium sulfide is used to treat ingrown-toenails. Another objective of the present invention is to soften these horny tissues for easy and speedy trimming and removal without pain or discomfort.

## SUMMARY OF THE INVENTION

This invention relates to a method to facilitate physical trimming and removing horny human tissues in a speedy and atraumatic fashion. Particularly, the invention includes a method of softening horny human tissues which comprises applying a topical composition comprising an effective amount of at least one sulfur containing compound to a human for a duration of time sufficient to soften. Further, the invention contemplates a method of removing horny human tissue which comprises applying a topical

composition comprising an effective amount of at least one sulfur containing compound to a human for a duration of time sufficient to soften and removing said horny tissue by a physical means. Still further the invention contemplates a kit which comprises a first topical composition which softens horny human tissue comprising an effective amount of at least one sulfur containing compound and a second topical composition comprising at least one active agent useful in the treatment of horny human tissue.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the pH effect of a solution containing 2% calcium thioglycolate and 10% urea on nail hydration (N=2).

Figure 2 shows that the pH dependency of nail hydration at pH>9.

Figure 3 is a graph showing the pH effect of 2% thioglycerol aqueous solutions with pH's ranging from pH 7 to pH 9.

Figure 4 is a graph plotting the percentage weight gain of nail clippings verses the solution pH for the 24-hour treatment using thioglycerol solutions.

Figure 5 is a graph showing the effect of different concentrations of calcium, sodium, and ammonium salts of thioglycolic acid, as well as the pH of calcium thioglycolate on nail hydration.

Figure 6 is a graph showing the nail hydration of several reducing agents other than thioglycolates.

Figure 7 shows the nail swelling profiles in three compositions containing calcium thioglycolate.

Figure 8 shows the miconazole nitrate permeation profiles through human nails under three experimental conditions.

## DETAILED DESCRIPTION OF THE INVENTION

This invention relates to methods to facilitate physical trimming and removing horny human tissues in a speedy and atraumatic fashion. Particularly, the invention includes a method of softening horny human tissues which comprises applying a topical composition comprising an effective amount of at least one sulfur containing compound to a human for a duration of time sufficient to soften. Further, the invention contemplates a method of removing horny human tissue which comprises applying a topical composition comprising an effective amount of at least one sulfur containing compound to a human for a duration of time sufficient to soften and removing said horny tissue by a physical means.

"Horny human tissue" is defined as the keratinous, and often hyper-proliferative tissues such as diseased nails, and hardened tissues of the skin such as corns and callus. Preferably the horny human tissues are diseased nails. Those tissues include but are not limited to the thickened diseased portions of the nail which is common in onychomycosis and psoriasis.

An "effective amount" of a sulfur containing compound refers to the amount which is necessary to soften horny human tissue. The amount ranges from about 0.1% to about 40%, preferably, from about 0.5% to about 20%, more preferably, from about 1% to about 10%.

The term "duration of time" refers to the time that is required to soften the horny human tissue. This time period is dependent on the sulfur compound used, its concentration, the type of horny human tissue, and other factors such as composition pH. In general, if horny human tissue is a nail, the duration of time may vary from about one minute to about one day, preferably between 5 minutes and 12 hours, more preferably between 10 minutes and 30 minutes. If the horny tissue is a corn or callus, the same time periods are preferred.

The "physical means" include, but are not, limited to filing, clipping, scraping, and other forms of manual mechanical abrasion. In addition, physical means can include mechanical abrasion with an electric grinding wheel or other electrically powered apparatus. However, the preferred physical means are manual mechanical abrasion without the use of an electrically powered apparatus.

According to the invention, the term "sulfur containing compound" refers to (a) thio-compounds with one or more sulfhydryl functional groups capable of reacting with disulfide bonds of keratin, (b) thio-containing amino acids and their derivatives, and (c) certain sulfides. The compositions of the invention include at least one sulfur containing compound, and if there is more than one sulfur containing compound, those compounds may be different chemical entities.

The thio-compounds include but are not limited to thioglycolic acid and its salts, such as glycolates of calcium, sodium, strontium, potassium, ammonium, lithium, magnesium, and other metal salts; thioethylene glycol, thioglycerol, thioethanol, as well as thioactic acid, thiosalicylic acid and their salts. The thio-containing amino acids and their derivatives are selected from a group consisting of L-cysteine, D-cysteine, DL-cysteine, N-acetyl-L-cysteine, DL-homocysteine, L-cysteine methyl ester, L-cysteine ethyl ester, N-carbamoyl cysteine, glutathion, and cysteamine. Certain sulfides, include but are not limited to calcium, sodium, potassium, lithium and strontium sulfides. The preferred thio-compounds with one or more sulfhydryl functional groups capable of reacting with disulfide bonds of keratin are the calicum and sodium salts of thioglycolic acid. The preferred thio-containing amino acids and their derivatives are L-cysteine and N-acetyl-L-cysteine. The preferred certain sulfides are sodium sulfide. The preferred sulfur containing compounds are thio-compounds with at least one sulhydryl functional groups capable of reacting with disulfide bonds of keratin.

Aside from the sulfur containing compounds, other substances, such as additional chemical agents, pharmaceutical excipients, and cosmetic agents may be included in the composition. The chemical agents include but are not limited to substances which aid the composition of the invention in softening the nail. Examples of such agents include but are not limited to urea and salicylic acid. The choice of which agent to use is dependent upon the identity of the sulfur containing compound. For basic sulfur containing compounds urea, is the additional chemical agent of choice. For acidic sulfur containing compounds, salicylic acid and urea are the additional chemical agents of choice. If additional chemical agents, such as urea are used, the concentration of urea to be used ranges from about 1% to about 50%, preferably from about 5% to 40%. If salicylic acid is used as an additional chemical agent, the concentration of salicylic acid to be used ranges from about 0.1% to about 40%, preferably from about 1 % to about 20%.

The cosmetic agents may include but are not limited to those agents which prevent potential skin irritation, such as emollients, vitamins (e.g., vitamin E) and herbal extracts (e.g., aloe vera). The pharmaceutical excipients include but are not limited to pH modifying agents such as pH-modifying agents (e.g., HCl, sodium hydroxide, and calcium hydroxide), organic solvents (e.g., propylene glycol, glycerol, etc.), cetyl alcohol, kaolin, talc, zinc oxide, titanium oxide, cornstarch, sodium gluconate, oils (e.g., mineral oil), ceteareth-20, ceteth-2, surfactants and emulsifiers, thickener (e.g., cellulose derivatives and gums), perfume, anti-oxidants, preservatives, and water. The choice of which pharmaceutical excipient to use is often controlled or affected by the type of sulfur containing compounds used in the composition. For example, when the sulfur containing compounds are thio-containing amino acids and their derivatives, preferably the pH of the composition is preferably below pH 7, and more preferably, below pH 5. Alternately, if the sulfur containing compound are thio-compounds and sulfides, the pH of the composition is preferably above pH 6, and more preferably, above pH 9.

The compositions of the present invention may be prepared in a number of forms which can be applied to horny human tissue. For example, the composition may be applied in a gel, cream, ointment, liquid, spray liquid, paint-/brush-on preparation, plaster, hydrogel-device. In addition, the composition may be applied with a bandage-like device. This device can be used to affix the composition to the horny human tissue and to provide desirable occlusion and protection from accidental wiping off. A device similar to that described by Sun et al in US Patent 5,696,164 may be used, and is hereby incorporated by reference. This device is constructed in such a way that during the softening process, only the horny human tissue is in direct contact with the softening composition. The contact between the composition and the surrounding skin tissues is minimized to prevent any skin irritation. Still further, the composition may be incorporated in an adhesive layer, which is coated on a pliable polymeric backing sheet. This device resembles an adhesive tape, only with the composition embedded in the adhesive layer. As another alternative, the softening compositions are incorporated in a hydrogel device. An aqueous solution or suspension of the softening composition are immobilized in a hydrogel layer, which is coated on a pliable polymeric backing sheet. During the application, the hydrogel layer will be situated over the tissue to be softened. The hydrogel device is secured to its position by the adhesion between the hydrogel layer and the horny human tissue and surrounding skin tissue. Alternatively, the hydrogel device is secured by an adhesive layer coated on the polymeric backing sheet at the edges of the hydrogel device in a configuration commonly known in the art as an "island-type bandage".

After softening, thinning and removal of the horny human tissue, a formulation containing at least one active agents for either therapeutic or cosmetic treatment of horny human tissue may be applied. Therefore, the invention contemplates a kit which comprises a first topical composition which softens horny human tissue comprising an effective amount of at least one sulfur containing compound and a second topical composition comprising at

least one active agent useful in the treatment of horny human tissue.

The terms "horny human tissue," "effective amount," "sulfur containing compound," "physical means" and "duration of time" are as described in the foregoing paragraphs.

As used herein, the term "active agents" refers drugs for treating diseased nails, nutrients or nail conditioners which may be used to improve damaged nails or maintain healthy nails, and nail growth promoters which may be used on damaged or healthy nails. More than one of these active agents may be present in the second topical composition. If more than one active agent is present, each agent may be different in chemical identity as well as function. These agents include but are not limited to antifungal agents, antibiotic agents, antibacterial agents, antipsoriatic agents, and antiinflammatory agents.

If the treated horny human tissue is a corn or callus and the underlying tissue is infected, those anti-fungal agents include but are not limited to miconazole, econazole, ketoconazole, itraconazole, fluconazole, bifoconazole, terconazole, butoconazole, tioconazole, oxiconazole, sulconazole, saperconazole, clotrimazole, undecylenic acid, haloprogin, tolnaftate, nystatin, ciclopirox olamine, terbinafine, amorolfine, naftifine, elubiol, griseofulvin, and their pharmaceutically acceptable salts. If antipsoriatic agents are needed, those agents include but are not limited to corticosteroids (e.g., betamethasone dipropionate, betamethasone valerate, clobetasol propionate, diflorasone diacetate, halobetasol propionate, amcinonide, dexamethasone, fluocinonide, fluocinolone acetonide, halcinonide, triamcinolone acetate, hydrocortisone, hydrocortisone verlerate, hydrocortisone butyrate, aclometasone dipropionate, flurandrenolide, mometasone furoate, methylprednisolone acetate), calcipotriene, anthraline, and their pharmaceutically acceptable salts. If antibacterial and antiseptic agents are required, such agents include but are not limited to mupirocin,

neomycin sulfate bacitracin, polymyxin B, *l*-ofloxacin, tetracyclines (chlortetracycline hydrochloride, oxytetracycline hydrochloride and tetrachcycline hydrochoride), clindamycin phsphate, gentamicin sulfate, benzalkonium chloride, benzethonium chloride, hexylresorcinol, iodines, methylbenzethonium chloride, phenol, quaternary ammonium compounds, triclocarbon, triclosan, tea tree oil, and their pharmaceutically acceptable salts. If the treated horny human tissue is a nail hardened by onychomycosis, the active agents include but are not limited to those described the agents listed in this paragraph as well as the agents listed in an earlier US patent application by Sun et al. (U.S. No.60/080,212). The aforementioned US patent application is hereby incorporated by reference.

The active agents may also be cosmetic agents, nutrients and minerals to improve the appearance or condition of the tissue underlying the horny human tissue. Such tissue includes but is not limited to the nail matrix, the nail bed or lower nail plate underneath a hardened nail plate as well as the skin below a callus or a corn. Cosmetic agents include but are not limited to pigments, dye, and other additives (e.g., silica, talk, zinc oxide, titanium oxide, clay powders. The formulation containing active agents may be in the form of a conventional topical formulation, e.g., as solution, gel, cream, lotion, ointment, rinse-off formulation, spray liquid, spray powder, aerosol, paint-on formulation such as lacquer/varnish and tincture. The formulation may be applied directly to the softened or thinned tissue, or applied in a bandage-like device, or in a hydrogel device similar to the methods described in the previous sections for softening horny human tissue. Optionally, a protective means, in the form of a polymeric coating, an adhesive-coated pliable tape, or a bandage is applied to the treated tissue to prevent the actives from leaching out and being washed off. As described previously, the compositions of the invention may delivered by a number of means and may contain chemical agents, and pharmaceutical excipients.

For example if the horny human tissue is a thick hardened nail plate

(due to onychomycosis, psoriasis, or other diseases), this nail plate may be treated with a topical composition of the invention, containing calcium thioglycolate as the sulfur containing compound for about 15 minutes. One such useful composition is the commercial depilatory, Nair® (Carter-Wallace, Inc., New York, listed ingredients include sodium thioglycolate, calcium thioglycolate, calcium hydroxide, water, mineral oil, ceteareth-20, fragrance and iron oxide). Generic commercial depilatories typically contains 5 to 7.5% calcium thioglycolate ("Cosmetic and Toiletry Formulations", 2nd Ed. Vol.2, pages 243 & 255, by E.W. Flick, Noyes Publicaitons, New Jersey, 1991). Therefore commercial depilatories containing the sulfur containing compounds of the invention may be used in the method of this invention. Since the depilatory-treated nails were less hard, the effort (specifically, the force needed) to file the nail was significantly reduced when the thioglycolate-containing composition was used to facilitate the nail thinning process. This finding has a practical importance, since it would be difficult, and might even be painful under certain circumstances, for a patient to file the disease-thickened nails to thin them for better efficacy of a topical treatment. It is particularly true for toenails, and for elderly patients among whom the nail fungal disease has a much higher infection rate than the younger population.

Immediately after softening with the sulfur containing composition, the previously hardened nail plate may be thinned by mechanical filing or scraping over the treated nail plate. Once a thinned nail plate is obtained, the remaining tissue may be treated with a nail lacquer containing an antifungal agent such as miconazole nitrate. Aside from the antifungal agent, this lacquer may also contains cosmetic ingredients, which include but are not limited to, pigments, dye, and other additives (e.g., silica, talk, zinc oxide, titanium oxide, clay powders) for improved mechanical properties and non-shiny appearance. In addition to or instead of the nail lacquer, other formulation forms such as antifungal containing, liquids, creams, ointments, liquid sprays and aerosols may also be used.

In order to illustrate the invention the following examples are included. These examples do not limit the invention. They are meant only to suggest a method of practicing the invention. Those knowledgeable in the treatment of horny human tissue as well as other specialties may find other methods of practicing the invention. However, those methods are deemed to be within the scope of this invention.

## EXAMPLES

### Example 1.

Nail Softening and Swelling When Exposed to Nail Softening Compositions

Human nails are hard and somewhat elastic under normal conditions. After a long-time soaking in water, the nail will become slightly swollen, less hard and more elastic, i.e., becoming "softer". It was found that the nail hardness and nail swelling are inversely proportional, i.e., the higher degree a nail swells in an aqueous solution, the softer it becomes. Therefore, the ability of a composition to soften the nail can be investigated by studying its ability to swell the nail. The nail swelling when exposed to compositions containing S-compounds was studied by in vitro experiments. The experimental procedure is briefly described. Clean human finger nail clippings were equilibrated to a constant weight by placing them in a desiccator over saturated $CaCl_2.6H_2O$ aqueous solution (29% relative humidity at room temperature) for at least 48 hours before use. Approximately 30 mg of nail clippings were weighed in a glass vial, the exact initial weight recorded. Three milliliters of a test vehicle, (pre-warmed to 32 °C) were added to the vial and maintained at 32 °C under stirring in a heating/Stirring Module (Reacti-Therm III®, Pierce). The changes in nail weight were monitored at predetermined intervals over a period of time. All the percentage values hereafter are by weight unless otherwise specified.

The pH effect of thio-compounds on nail softening and nail hydration was studied. The results were shown in Figures 1 and 2. For the experiment shown in Figure 1, all the testing solutions contained 2% calcium thioglycolate

and 10% urea. Urea was used here to assist thioglycolate to break the disulfide bonds of nail protein. From pH 7 to pH 9, only about 150% nail hydration could be achieved. At the higher pH's, i.e., with more alkaline solutions, the nail hydration increased to 229% at pH 10 and to 258% at pH 11. The pH dependency of nail hydration at pH>9 can be seen more clearly when the results were plotted with the percentage weight gain of nail clippings verses the solution pH (Figure 2). There is a linear increase in nail hydration, as a result of the treatment with 2% calcium thioglycolate and 10% urea aqueous solutions from pH 9 to pH 11. This finding indicates that the preferred pH range to enhance nail absorption is equal to and above pH 10.

The pH effect of a thio-compound other than thioglycolate was investigated using 2% thioglycerol aqueous solutions with pH's ranging pH 7 - pH 10 (Figure 3). There was a slight increase in nail hydration as the solution pH increased from pH 7 to pH 9. Similar to the nail hydration enhanced by calcium thioglycolate in Figures 1 & 2, a steep increase in nail hydration occurred at pH>9 (Figure 4).

Figure 5 compares the nail hydration effect of calcium, sodium and ammonium salts of thioglycolic acid of various concentrations at pH 10. Nail hydration in water was included as a control. The result for 1% calcium thioglycolate at pH 11 was also included. It can be seen that at the same thioglycolic acid concentration (e.g., 2%) and pH condition, sodium salt and ammonium salt appears to have a greater nail hydration capability than calcium salt. In general, nail hydration increased as the thioglycolate concentration increased, and as the pH of calcium thioglycolate solution (1%) increased (from pH 10 to pH 11).

Figure 6 shows the nail hydration results from several non-thioglycolate reducing agents tested. Among them, thioethylene glycol, thiolactic acid and thioglycerol of 2% at pH 10 were found to be able to produce significant nail hydration. The rank order of nail hydration enhancement capability of the

tested chemicals (from best to worse) at 2% concentration and pH 10 is: thioethylene glycol > thiolactic acid > thioglycerol > sodium sulfide > thiosalicylic acid > sodium sulfite > sodium bisulfite > sodium thiosulfate = thiourea. Sodium thiosulfate and thiourea, which do not have any sulfhydryl functional group in their structure, were included in the nail hydration experiment to demonstrate the importance of sulfhydryl groups for the purpose of nail penetration enhancement. As can be seen from the data, the absence of sulfhydryl groups in sodium thiosulfate and thiourea made them completely ineffective in cleaving keratin disulfide bonds, as indicated by the poor nail hydration. For the same reason, 10 percent ascorbic acid did not increase nail hydration as compared to water.

Figure 7 shows the nail swelling profiles in three compositions containing calcium thioglycolate. Extensive swelling of nail clippings was observed with all the compositions tested during the 4-hour experiment. The composition containing 5% calcium thioglycolate in distilled water showed a lower nail swelling than the composition with 5% calcium thioglycolate, 20% urea in water. The commercial Nair® lotion (hereinafter "commercial depiliatory," Carter-Wallace, Inc., New York, the listed ingredients include sodium thioglycolate, calcium thioglycolate, calcium hydroxide, water, mineral oil, ceteareth-20, fragrance and iron oxide) behaved somewhat between the two compositions, i.e., initially similar to the former, and later similar to the latter.

Example 2.

Effect of Nail Softening Compositions on Nail Thinning Process and Subsequent Drug Permeation

The nail swelling results in the earlier example suggest that certain sulfur containing compositions will cause rapid and significant nail softening. It is often desirable to make a thick nail thinner by filing prior to the topical treatment because a thinner nail presents a short pathway and hence less resistance to the topically applied drug. One can take advantage of the nail softening effect of sulfur containing compositions to facilitate the nail thinning

process and to promote drug penetration through the nail. A commercial depilatory lotion comprising a sulfur containing composition was used to illustrate this approach. The depilatory lotion used in the experiments was the commercial depilatory. The listed ingredients include sodium thioglycolate, calcium thioglycolate, calcium hydroxide, water, mineral oil, ceteareth-20, fragrance and iron oxide.

An in vivo test was conducted on two volunteers to examine whether this composition could cause nail softening, thus making it easier to thin the nails. A layer of the depilatory lotion was applied the finger nails of the volunteers. No uncomfortable sensation or irritation was felt. After 15 minutes, the depilatory lotion was washed off with water and the treated nails were examined. The superficial part of the treated nail turned white and soft. This layer of significantly hydrated and softened nail tissue could easily be scraped off.

Example 3

Drug Permeation Though Nails

The following experiments were carried out to test drug permeation through the nail under in vitro conditions. Thick nail plates from toes and thumbs of cadaver were used in the study. These thick nails, most of them with the original thickness around 1 mm or greater, were filed down at the dorsal nail surface to a thickness between 0.4 mm and 0.6 mm, which is the thickness of typical fingernails. Similar to the earlier in vivo test, a group of the nails were first treated with a layer of the commercial depilatory lotion on the dorsal nail surface at root temperature for 15 minutes. The depilatory treated nail plates were then thinned by filing. It was found that filing the depilatory-treated nails was significantly easier than filing the nails which were not treated with the depilatory lotion. It took less than half of the time to file the nail to the required thickness for a depilatory treated nail, as opposed to the nail without nail-softening treatment. The average length of time needed to thin by filing a depilatory-treated nail was 30 seconds to one minute.

The experimental procedures of drug penetration through the nail are described as following. The human nail plates were treated under three different conditions A, B, and C (N=3 for A&B, N=2 for C). Under condition A, thick nail plates from thumbs were mechanically filed down to the range of 0.4-0.6mm (a thickness comparable to average human fingernails). In condition B, the nail plates were mechanically filed down as in condition A. In condition C, the nail plate was treated for 15 minutes with the sulfur containing depilatory of example 2 and mechanically thinned to a range of 0.4-0.6 mm. The thinned nail plates of conditions A, B, and C were mounted in nail diffusion cells (similar to the Franz diffusion cells) with the dorsal nail side facing the donor cell, and the ventral nail side in contact with the receptor fluid, pH 4 phosphate-citrate buffer. A nail enhancer formulation containing 10% n-acetylcysteine (NAC) and 20% urea in distilled water (100 micro-liters) was placed in the donor cells to pretreat the nail plate of B & C for 8 hours. After the enhancer formulation was removed from the donor cells, 50 micro-liters of a nail lacquer formulation containing 5% miconazole nitrate was applied to form a thin layer on the dorsal nail plate in the donor cells of A, B, and C. The composition of the miconazole nail lacquer is shown in Table 1. At the end of one week from starting of the permeation experiment, the nail lacquer was removed with ethanol swabs. An aliquot of the sample was taken from the receptor fluid, and replaced with fresh buffer. The treatment with the nail enhancer and the antifungal treatment were repeated. At the end of the second week, the nail permeation experiment was terminated. The amount of drug penetrated through the nail plate was determined by analyzing the receptor fluid with high pressure liquid chromatography (HPLC). The drug content in the nail plates was determined by removing surface-bound drug with alcohol swabs, digesting the nail plates, and analyzing the drug with HPLC. The experiment was conducted in replicates for a total length of two weeks at 32 °C.

The drug permeation profiles in Figure 8 show that miconazole nitrate

in the nail lacquer was able to penetrate through the nail plates to reach the receptor side. The drug permeation was significantly higher through the nail plates pretreated with nail penetration enhancers (Conditions B & C), than the nails without penetration enhancer pretreatment (Condition A). This result has an importance implication. The pathological fungi responsible for onychomycosis (i.e., dermatophytes and Candida albicans) are known to hide in the nail bed, which is a main reason that topical treatments have not been efficacious because of the difficulty to deliver therapeutically sufficient quantity of antifungal drugs across the nail plate to nail bed. The complete nail permeation results are tabulated in Table 2. It can be seen that a small amount of miconazole nitrate could penetrate through the nail plate when the thick toe/thumb nail was mechanically filed down to the thickness comparable to typical fingernails (Condition A). The fact that only a very small percentage of the drug delivered into the nail could be recovered from the receptor side indicates that majority of the drug is likely still entrapped in the nail tissue close to the dorsal surface. The drug concentration within the nail might decline rapidly as the distance increased from the dorsal surface, the drug application site.

Nail filing followed by pretreatment with nail penetration enhancers (Condition B) significantly increased the amount of the drug penetrating through the nail. The minimal inhibition concentration, corresponding to the threshold concentration for antifungal effect, for miconazole nitrate is reported to be from about 0.1to about 1.0 mic.g/cm3. The quantity of miconazole nitrate delivered in vitro into and through the nail plates in Condition B is very likely sufficient to be efficacious if applied clinically. Softening nail before mechanical nail filing, followed with nail penetration enhancer pretreatment (Condition C) also delivered a significant amount of miconazole nitrate into and through the nail. The most important implication which can be derived from the result in the Condition C, is that the nail softening procedure with the calcium thioglycolate lotion significantly reduces the time and effort for the nail thinning process with filing. This brings a real benefit to patients, especially to

those who lack the body flexibility and precision limb control for the task because of their advanced age or poor physical conditions.

TABLE 1.

The composition of antifungal nail lacquer used in the study

| INGREDIENT | PARTS BY WEIGHT |
|---|---|
| Miconazole Nitrate, USP/NF | 5.0 |
| Ascorbyl Palmitate, USP/NF | 0.1 |
| Concentrated Hydrochloric Acid, USP/NF (37%) | 2.5 |
| Isopropyl Myristate, USP/NF | 1.0 |
| Ethyl Alcohol, 200 proof, SDA 40B | 36.4 |
| Ethyl Acetate, USP/NF | 40.0 |
| Carboset 525 acrylate polymer | 15.0 |
| | Total: 100 |

TABLE 2.

Results of 14-day Study of In Vitro Miconazole Nitrate Penetration through the Nail (Average ± Standard Deviation)

| NAIL PENETRATION EXPERIMENT CONDITIONS Big Toe and Thumb Nails | | DRUG REACHED RECEPTOR (mcg/cm2) | DRUG RETAINED IN THE NAIL (mcg/cm2) |
|---|---|---|---|
| A | Filing alone (N = 3) | 0.23 ± 0.35 | 238.9 ± 24.8 |
| B | (Filing) + (8-hour enhancer pretreatment) (N = 3) | 24.2 ± 26.8 | 105.9 ± 38.1 |
| C | (15-min Nail Softening) + (Filing) + (8-hour enhancer pretreatment), (N = 2) | 44.3 | 374.6 |

- mcg= Microgram

What is claimed is:

1. A method of softening horny human tissues comprising applying a topical composition comprising an effective amount of at least one sulfur containing compound to a human for a duration of time sufficient to soften.

2. The method of claim 1 wherein said horny human tissue is a diseased nail.

3. The method of claim 1 wherein said at least one sulfur containing compound is a thio-compounds with one or more sulfhydryl function groups capable of reacting with disulfide bonds of nail keratin.

4. The method of claim 1 wherein said at least one sulfur containing compound is selected from the group consisting of thioglycolic acid, calcium thioglycolate, sodium thioglycolate, strontium thioglycolate, potassium thioglycolate, ammonium thioglycolate, lithium thioglycolate, magnesium thioglycolate, thioethylene glycol, thioglycerol, thioethanol, thioacetic acid, and thiosalicylic acid.

5. The method of claim 1 wherein said at least one sulfur containing compound is selected from the group consisting of thioglycolic acid, calcium thioglycolate, sodium thioglycolate, strontium thioglycolate, potassium thioglycolate, ammonium thioglycolate, lithium thioglycolate, and magnesium thioglycolate.

6. The method of claim 1 wherein said at least one sulfur containing compound is a thio-containing amino acids and their derivatives.

7. The method of claim 1 wherein said at least one sulfur containing compound is selected from the group consisting of L-cysteine, D-cysteine, DL-cysteine, N-acetyl-L-cysteine, DL-homocysteine, L-cysteine methyl ester, L-cysteine ethyl ester, N-carbamoyl cysteine, glytathion, and cysteamine.

8. The method of claim 1 wherein said at least one sulfur containing compound is selected from the group consisting of L-cysteine and N-acetyl-L-cysteine.

9. The method of claim 1 wherein said at least one sulfur containing compound are certain sulfides.

10. The method of claim 1 wherein said at least one sulfur containing compound is selected from the group consisting of potassium sulfide, calcium sulfide, sodium sulfide, lithium sulfide and strontium sulfide.

11. The method of claim 1 wherein said effective amount of sulfur containing compound is from about 0.1% to about 40 %.

12. The method of claims 6, 8, or 11 wherein said effective amount of sulfur containing compound is from about 0.5% to about 20%.

13. The method of claims 6, 8, or 11 wherein said effective amount of sulfur containing compound is from about 1% to about 10 %.

14. The method of claim 1 wherein said duration of time is from about 1 minute to about 1 day.

15. The method of claim 1 wherein said duration of time is from about 5 minutes to about 12 hours.

16. The method of claim 1 wherein said duration of time is from about 10 minutes to about 30 minutes.

17. The method of claim 1 wherein said at least one sulfur containing compound is selected from the group consisting of thioglycolic acid, calcium thioglycolate, sodium thioglycolate, strontium thioglycolate, potassium thioglycolate, ammonium thioglycolate, lithium thioglycolate, and magnesium thioglycolate, said effective amount is about 1% to about 10%, and said duration of time is from about 10 minutes to about 30 minutes.

18. A method of removing horny human tissue comprising applying a composition comprising an effective amount of at least one sulfur containing compound to a human for a duration of time sufficient to soften and removing said horny tissue by a physical means.

19. The method of claim 19 wherein said physical means is manual mechanical abrasion.

20. The method of claim 20 wherein said physical means is scraping with a metal file, a plastic file, a wooden file, emery board, or a human fingernail.

21. The method of claim 20 wherein said at least one sulfur containing compound is selected from the group consisting of thioglycolic acid, calcium thioglycolate, sodium thioglycolate, strontium thioglycolate, potassium thioglycolate, ammonium thioglycolate, lithium thioglycolate, and magnesium thioglycolate and the duration of time is from about 10 minutes to about 30 minutes.

22. A kit which comprises a composition which softens horny human tissue comprising an effective amount of at least one sulfur containing compound and at least one active agent useful in the treatment of horny human tissue.

23. The kit of claim 23 wherein the horny human tissue is a diseased human nail.

24. The kit of claim 23 wherein said at least one active agent is selected from the group consisting of miconazole, econazole, ketoconazole, itraconazole, fluconazole, bifoconazole, clotrimazole, amorolfine, tolnaftate, ciclopirox olamine, terbinafine, amorolfine, and elubinol.

25. The kit of claim 23 wherein said at least one active agent is selected from the group consisting of betamethasone dipropionate, betamethasone valerate, clobetasol propionate, diflorasone diacetate, halobetasol propionate, amcinonide, desoximetasone, fluocinonide, fluocinolone acetonide, halcinonide, triamcinolone acetate, hydrocortisone, hydrocortisone verlerate, hydrocortisone butyrate, aclometasone dipropionate, flurandrenolide, mometasone furoate, methylprednisolone acetate, calcipotriene, anthraline, and their pharmaceutically acceptable salts.

26. The kit of claim 23 wherein said at least one active agent is selected from the group consisting of mupirocin, neomycin sulfate bacitracin, polymyxin B, *l*-ofloxacin, tetracyclines (chlortetracycline hydrochloride, oxytetracycline hydrochloride and tetrachcycline hydrochoride), clindamycin phsphate, gentamicin sulfate, benzalkonium chloride, benzethonium chloride, hexylresorcinol, iodines, methylbenzethonium chloride, phenol, quaternary ammonium compounds, triclocarbon, triclosan, tea tree oil, and their pharmaceutically acceptable salts.

27. The kit of claim 23 wherein said at least one sulfur containing compound is selected from the group consisting of thioglycolic acid, calcium thioglycolate, sodium thioglycolate, strontium thioglycolate, potassium thioglycolate, ammonium thioglycolate, lithium thioglycolate, and magnesium thioglycolate, said effective amount is about 1.0% to about 10%, and said duration of time is from about 10 minutes to about 30 minutes.

FIG. 1

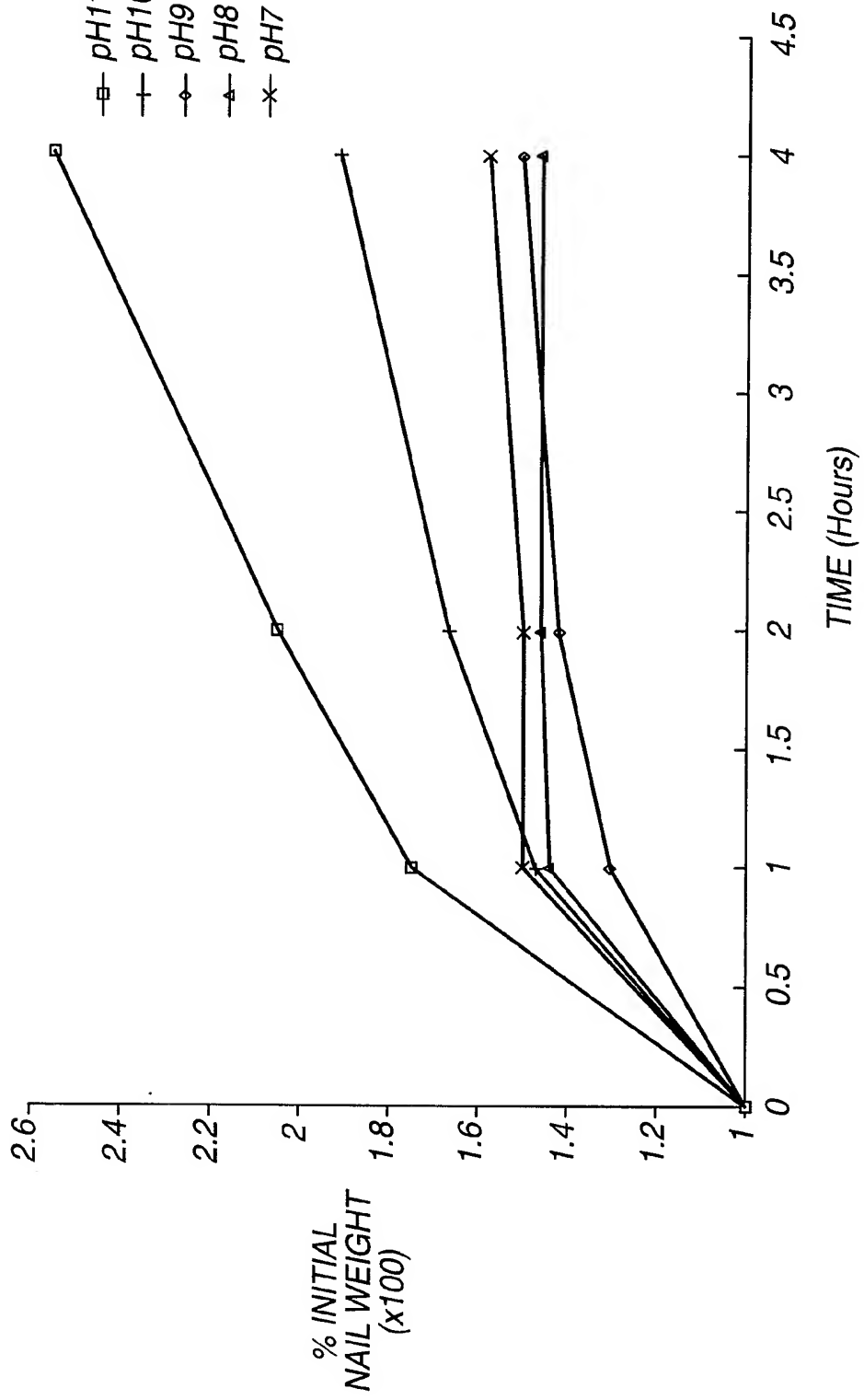

pH11
pH10
pH9
pH8
pH7
% INITIAL NAIL WEIGHT (x100)
TIME (Hours)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5

FIG. 2

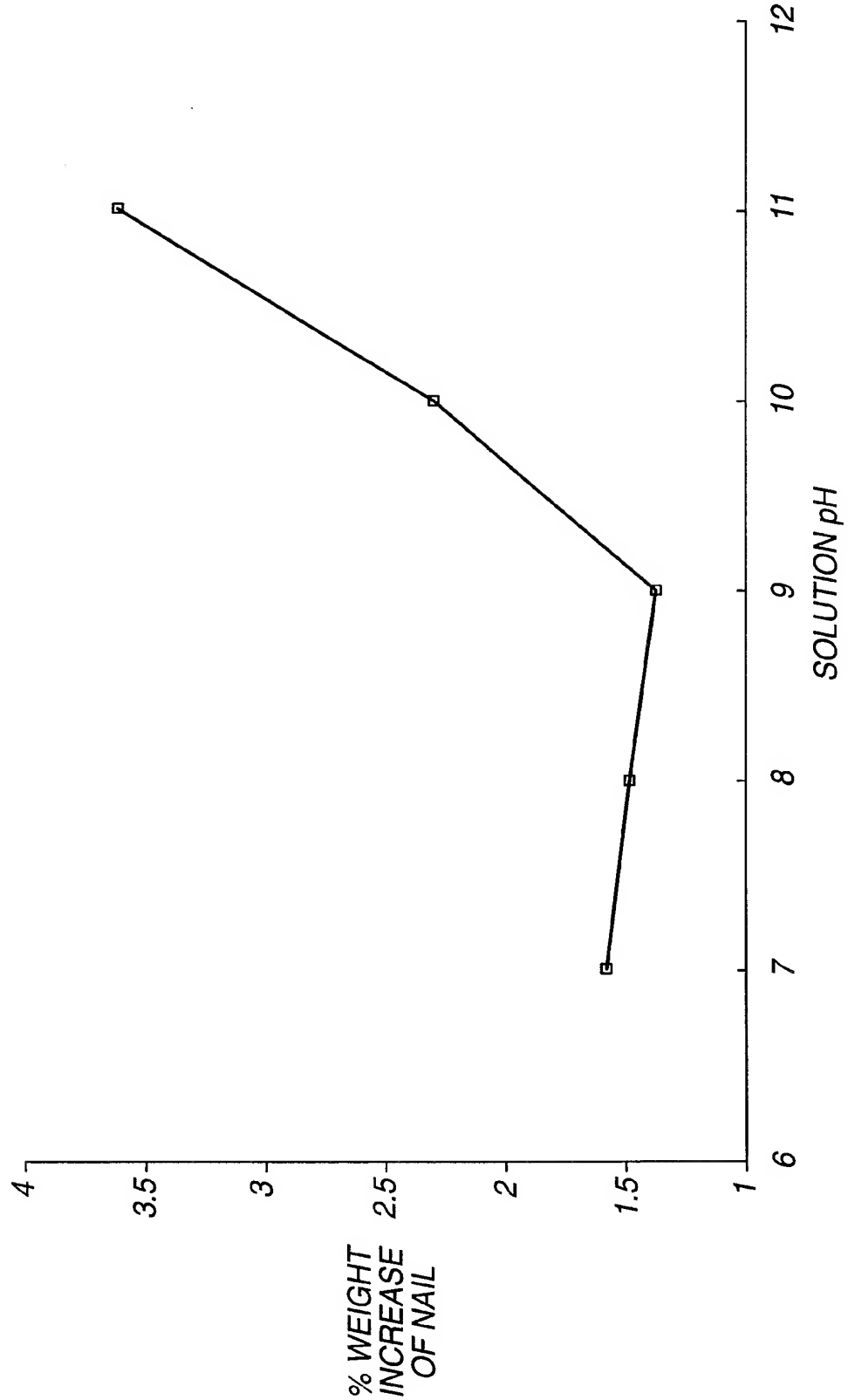

% WEIGHT INCREASE OF NAIL
4
3.5
3
2.5
2
1.5
1
6
7
8
9
10
11
12
SOLUTION pH

FIG. 3

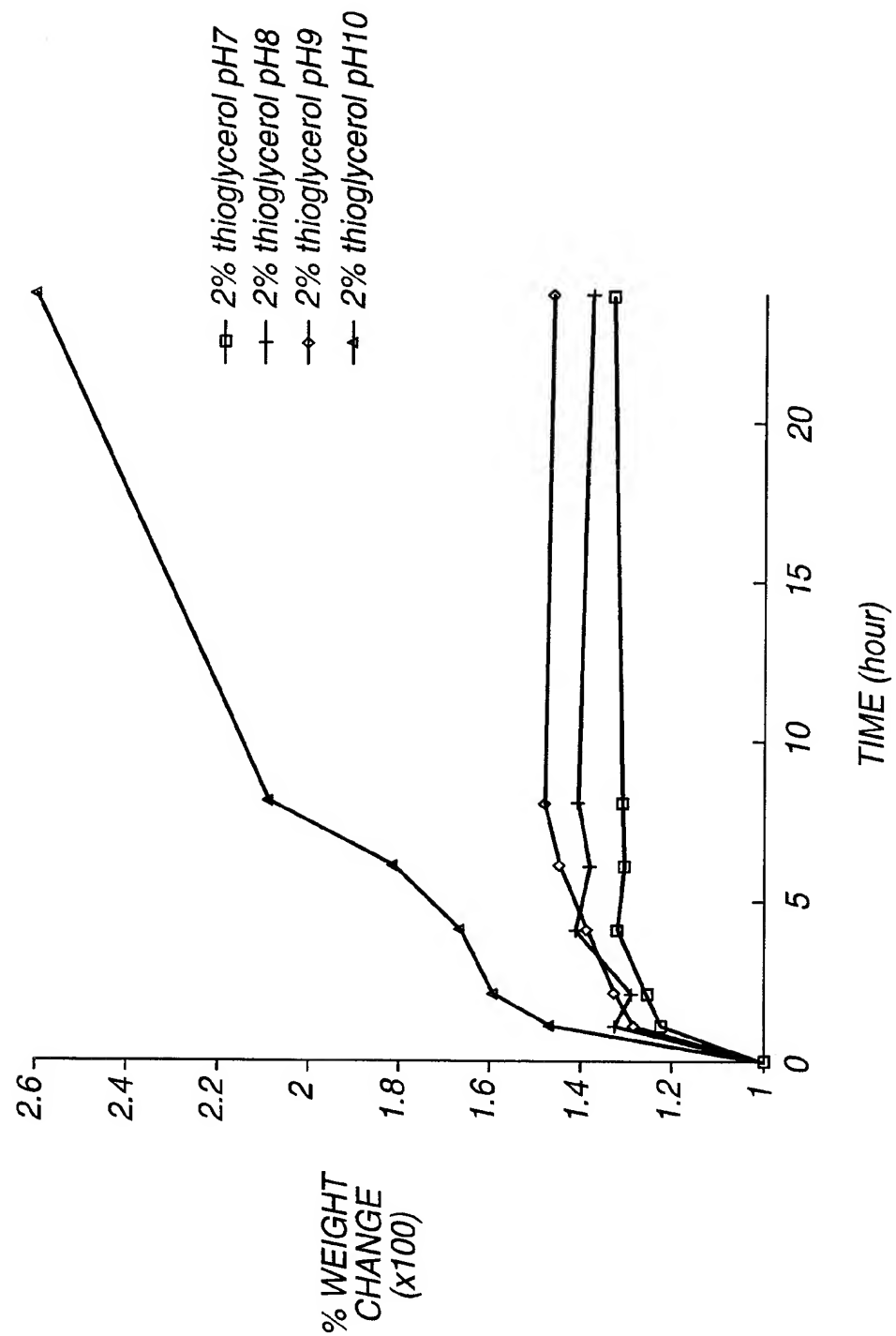

2% thioglycerol pH7
2% thioglycerol pH8
2% thioglycerol pH9
2% thioglycerol pH10
% WEIGHT CHANGE (x100)
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0
5
10
15
20
TIME (hour)

FIG. 4

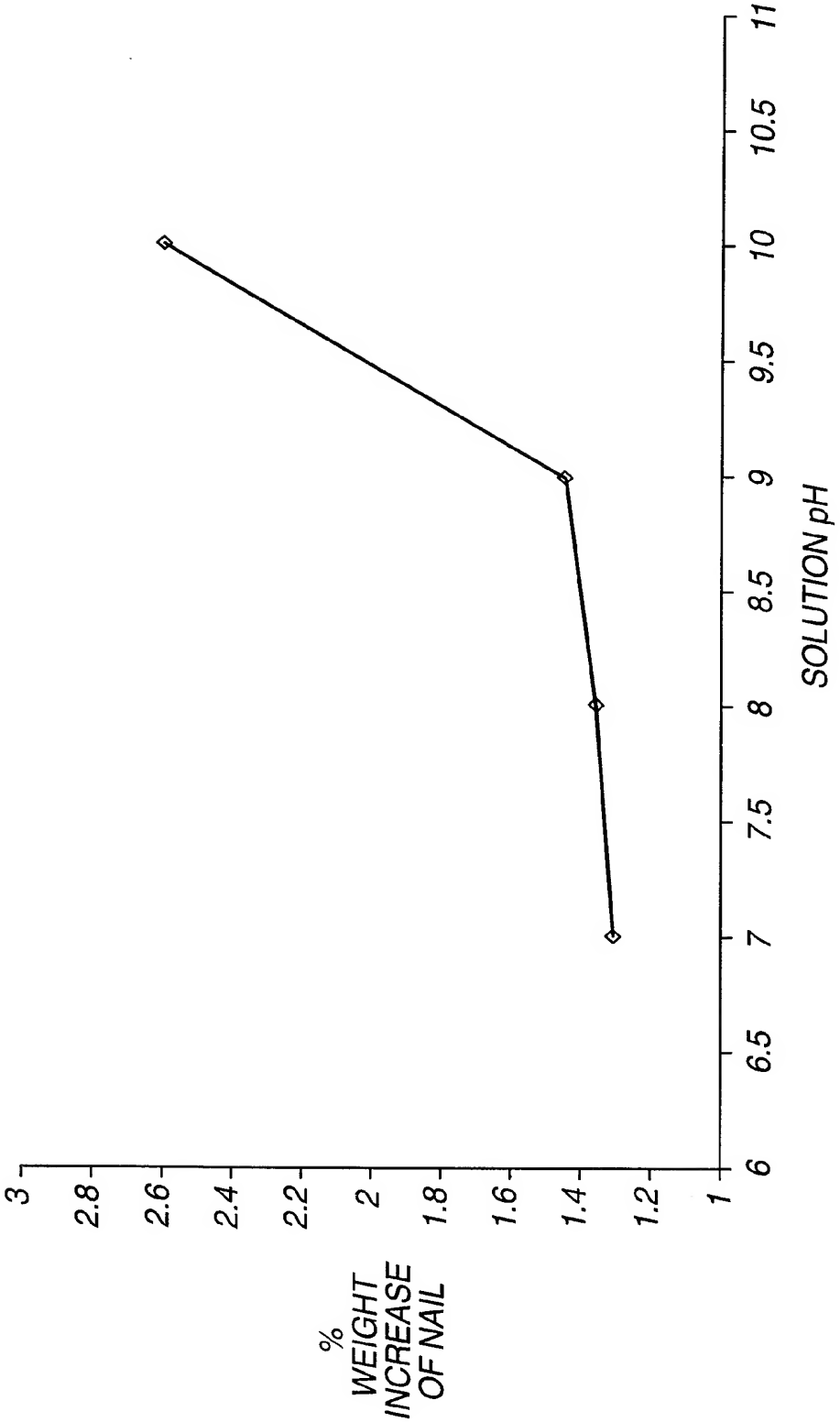

% WEIGHT INCREASE OF NAIL
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
6
6.5
7
7.5
8
8.5
9
9.5
10
10.5
11
SOLUTION pH

FIG. 5

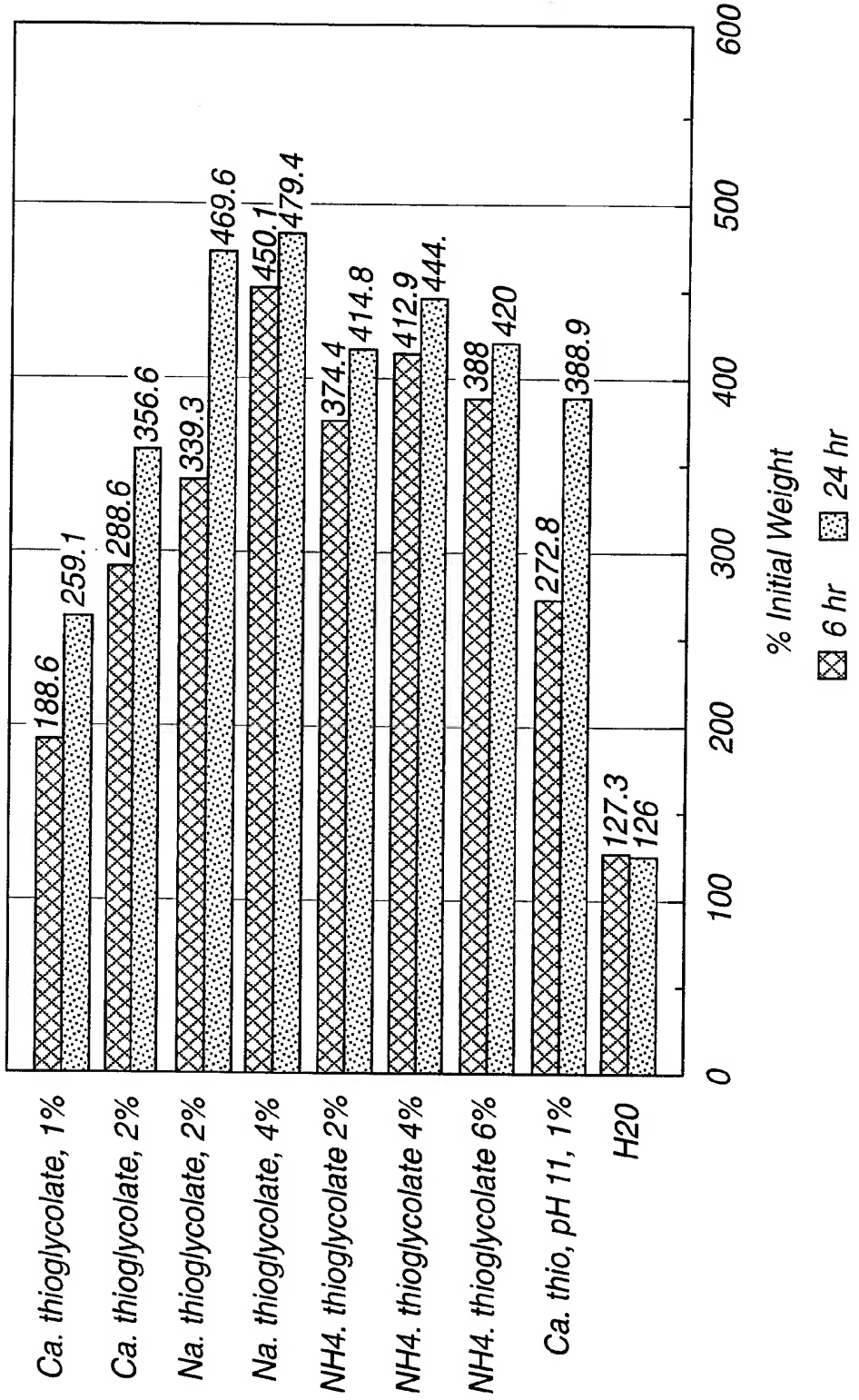

Ca. thioglycolate, 1%
Ca. thioglycolate, 2%
Na. thioglycolate, 2%
Na. thioglycolate, 4%
NH4. thioglycolate 2%
NH4. thioglycolate 4%
NH4. thioglycolate 6%
Ca. thio, pH 11, 1%
H20
188.6
259.1
288.6
356.6
339.3
469.6
450.1
479.4
374.4
414.8
412.9
444.
388
420
272.8
388.9
127.3
126
0
100
200
300
400
500
600
% Initial Weight
6 hr
24 hr

FIG. 6

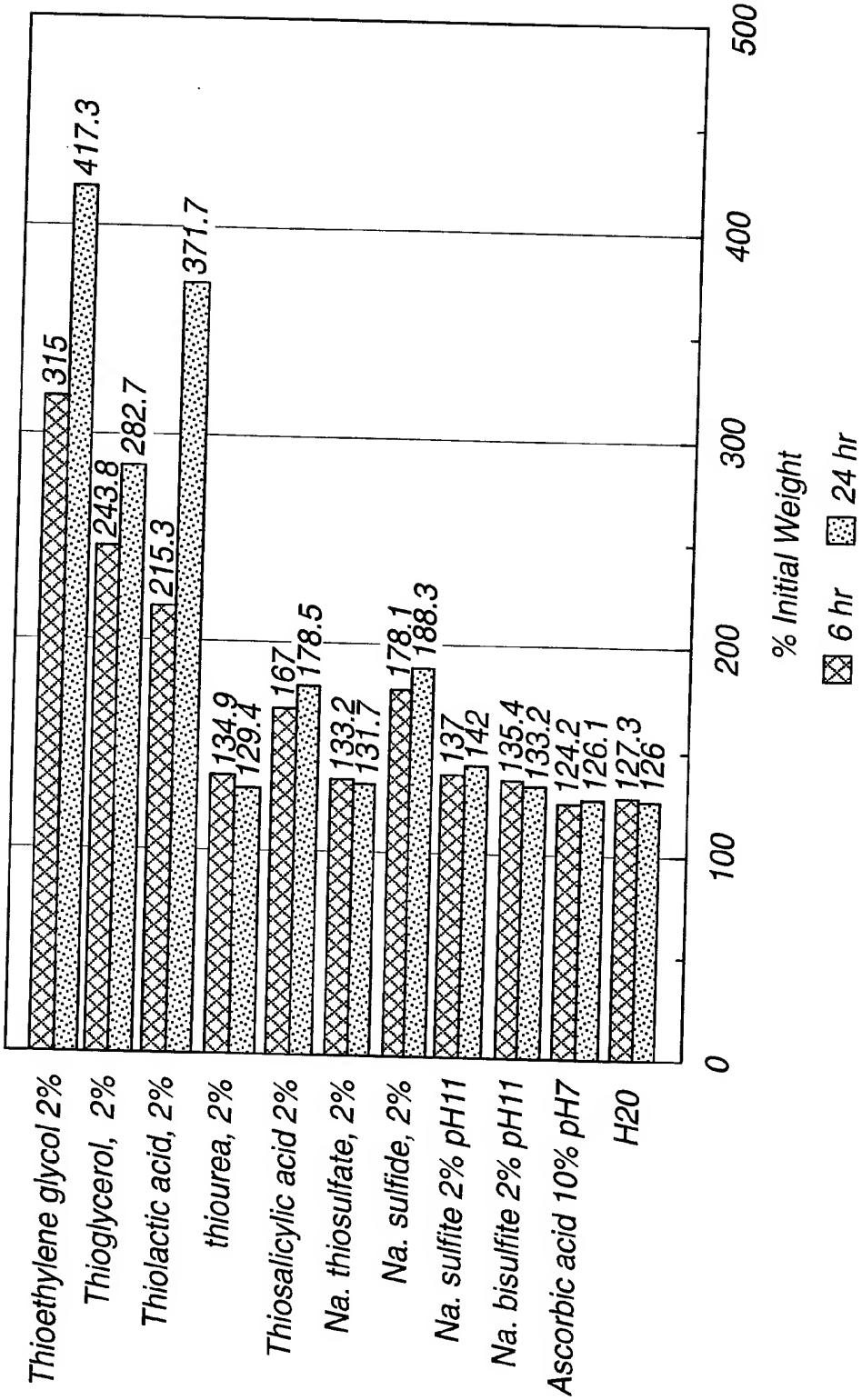

Thioethylene glycol 2%
Thioglycerol, 2%
Thiolactic acid, 2%
thiourea, 2%
Thiosalicylic acid 2%
Na. thiosulfate, 2%
Na. sulfide, 2%
Na. sulfite 2% pH11
Na. bisulfite 2% pH11
Ascorbic acid 10% pH7
H20
315
417.3
243.8
282.7
215.3
371.7
134.9
129.4
167
178.5
133.2
131.7
178.1
188.3
137
142
135.4
133.2
124.2
126.1
127.3
126
0
100
200
300
400
500
% Initial Weight
6 hr
24 hr

FIG. 7

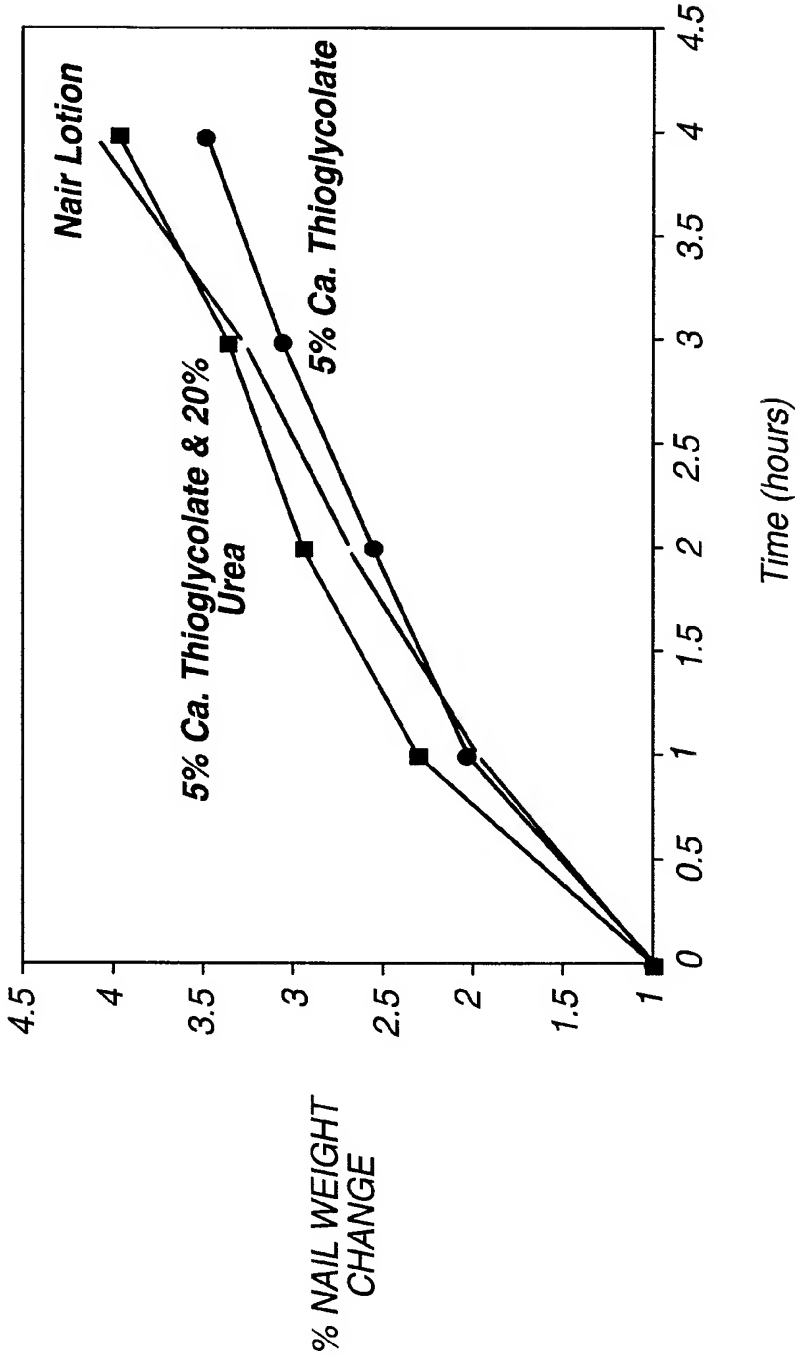
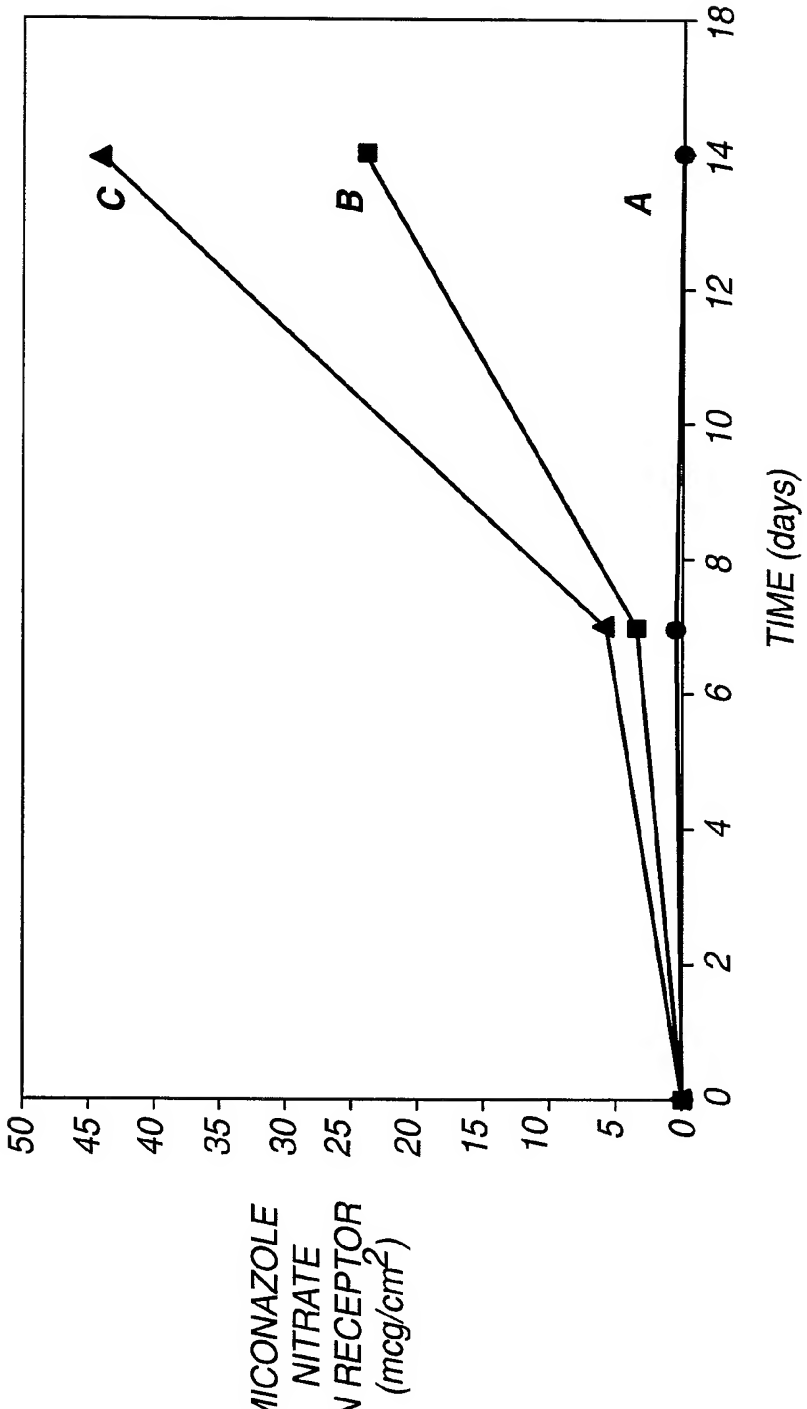

Nair Lotion
5% Ca. Thioglycolate & 20% Urea
5% Ca. Thioglycolate
% NAIL WEIGHT CHANGE
Time (hours)
4.5
4
3.5
3
2.5
2
1.5
1
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5

FIG. 8


MICONAZOLE NITRATE IN RECEPTOR (mcg/cm2)
0
5
10
15
20
25
30
35
40
45
50
TIME (days)
0
2
4
6
8
10
12
14
18
A
B
C

# INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 00/08267

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K7/04 A61K7/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 94 16991 A (SCHERING-PLOUGH HEALTHCARE PRODUCTS) 4 August 1994 (1994-08-04) claims 1-3,10 page 4, line 22 -page 5, line 4 example 3 | 1,2, 9-16,18 |
| X | EP 0 440 298 A (GIST-BROCADES) 7 August 1991 (1991-08-07) cited in the application claims 1-4,12,13 page 3, line 8-31 | 1-3,6-8, 11-13 |
| X | DE 10 00 567 B (HANS FASCHING ERBEN) 10 January 1957 (1957-01-10) claim 1 page 1, column 1 | 1-5, 11-13 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 August 2000 | 24/08/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL – 2280 HV Rijswijk<br>Tel. (+31–70) 340–2040, Tx. 31 651 epo nl,<br>Fax: (+31–70) 340–3016 | Peeters, J |

1

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. tional Application No
PCT/US 00/08267

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